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MASS CULTURE OF MACROCENTRUS ANCYLIVORUS AND ITS HOST, THE POTATO TUBER MOTH^{1, 2}

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INTRODUCTION

AN INSECT larva collected in Orange County, California, September 30, 1942, in the course of routine inspection work by agricultural officials, proved to be that of the oriental fruit moth, *Grapholitha molesta* (Busck). The discovery that this destructive insect had become established in California immediately created a situation which required the concerted action of entomologists and state agricultural officials. On the basis of earlier studies concerning the behavior and economic importance of this insect in other regions, the assumption seemed reasonable "that it would be a serious pest of deciduous fruit in California, particularly in view of the extensive production here of late canning peaches" (Smith, Essig, *et al.*, 1933).⁶ The California State Legislature within a few months appropriated for use of the California Department of Agriculture over \$800,000 for the eradication of the moth if surveys indicated eradication to be practical, and for research involving biological and chemical control (Mackie, 1944).

The surveys showed, however, that the moth was so widespread in the peach-growing areas that the proposed eradication campaign was inadvisable.

The program then resolved itself into retarding the spread of the moth into uninfested districts, the development of methods of control for use when control became necessary, and the introduction and release of insect enemies.

A part of the policy of trying to delay spread of the pest was the program of mass production and colonization of the parasite *Macrocentrus ancylivorus* Roh. in those areas revealed by the survey to be infested, since *Macrocentrus* had proved to be the most effective control of the oriental fruit moth in eastern states. The parasite appears to be an efficient searcher and it was felt to be sound policy to keep a population of *Macrocentrus* present at all times in the

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⁶ See "Literature Cited" at the end of this paper for full data on citations, which are referred to in the text by author and date.

infested orchards with the idea that they would discover and destroy a large portion of the twig-infesting larvae of the pest. *Macrocentrus* apparently disperses readily. It has been shown that the adult parasites are able to travel a distance of 6 miles (Driggers, 1941; Steenburgh, 1930).

The introduction into California of the various parasites of the moth known to occur in foreign countries was also considered an important part of the biological control program. This phase of the work, however, depends on the resumption of normal traveling conditions to the oriental-fruit-moth habitats in Japan and China and on the degree to which the moth becomes a pest in California.

PREVIOUS METHODS OF UTILIZING MACROCENTRUS AND RESULTS

The oriental fruit moth was first discovered in the United States in 1915 near Washington, D.C., probably having been introduced about 1913. Within a few years it had become very destructive to peaches, particularly the fruit. Attempts to control it by insecticides proved unsuccessful. As early as 1917, however, a native parasite was observed to be playing an important part in the natural control of the moth (Wood and Selkregg, 1918). Prior to 1929 this parasite, described by Rohwer (1923) as *Macrocentrus ancyliivorus*, was known to be parasitic on the oriental fruit moth only in a narrow coastal strip from southern Connecticut to southern Virginia (Allen, 1932). Here it had clearly demonstrated its value by destroying as high as 90 per cent of the twig-infesting larvae of the fruit moth. In New Jersey this parasite effected a decided reduction in the larvae infesting the fruit of the midseason varieties of peach (Allen, 1932). In Delaware during the ten-year period from 1931 to 1940, inclusive, the yearly average parasitism of the fruit moth ranged from 45 per cent in 1935 to 76.6 per cent in 1940. *Macrocentrus ancyliivorus* made up 92.7 per cent of all the parasitic species noted. It is credited with the prevention of fruit losses in excess of 10 per cent (Stearns and Amos, 1941).

As the oriental fruit moth spread westward from the Atlantic seaboard, the United States Bureau of Entomology and Plant Quarantine in coöperation with various state agencies followed the dispersal of the fruit moth with liberations of *Macrocentrus*. The Bureau's Moorestown laboratory, situated in the area where *Macrocentrus* was most readily collected, served remarkably well as the source of material.

In 1928 and 1929 experiments by Driggers (1930) demonstrated that parasitism by *Macrocentrus* could be increased by the liberation of relatively few adult parasites. In 1930 mass propagation of *Macrocentrus* was undertaken in Connecticut (Garman and Brigham, 1933) using the oriental fruit moth as host. The host and parasite were propagated on sliced green apples, which permitted propagation during the winter. The apples were thinnings picked during the summer and held in cold storage. In 1932 and 1933, 12,000 *Macrocentrus* were liberated in Connecticut peach orchards.

Then in New York a method was developed which permitted the production of 26,000 *Macrocentrus* in one season. According to Daniel (1936) the procedure was to build up an infestation of the larvae of the strawberry leaf roller *Ancyliis comptana fragariae* (Walsh and Riley) in large field cages and then

to introduce fertilized females of *Macrocentrus*. The parasitized host larvae were collected at the time of cocoon formation and stored in rooms where the emerging parasites were easily collected. A tenfold increase of female *Macrocentrus* was obtained by this method. The number of female *Macrocentrus* that can be produced from an acre of strawberries under cage is enough for liberation over 1,200 acres of orchard at the rate of about 500 females per acre (Allen, 1942).

Brunson and Allen (1944) concluded that in New Jersey periodic colonizations of *Macrocentrus* on second-brood larvae of the oriental fruit moth increase the parasitism of such larvae and consequently effect a reduction in fruit injury by about 50 per cent. Obviously a greater reduction should be obtained if the parasite is released against the first brood. With the methods just outlined, however, it was difficult to obtain *Macrocentrus* for colonization earlier than the middle of June.

The experiences of eastern entomologists had shown that the oriental fruit moth and the strawberry leaf roller, hosts that are attacked under natural conditions, were not adaptable to continuous mass propagation. Garman and Brigham (1933) stated that "production of *Macrocentrus* on the same scale as *Trichogramma* is impossible at present." Economical year-round production of *Macrocentrus* waited on the utilization of some host that would be continually available, as was the case in the mass production of *Trichogramma* (Flanders, 1930).

Stearns (1930) stated that parasitism has not and probably never will provide an adequate control for the oriental fruit moth. Nevertheless it was obvious that the first step in undertaking the control of the oriental fruit moth in California by biological means was to attempt the utilization of *Macrocentrus ancyllivorus*.

DEVELOPMENT OF MACROCENTRUS PRODUCTION IN CALIFORNIA

As the result of the distributional work of the Moorestown laboratory, *Macrocentrus* had become established on the Pacific Coast prior to 1938, apparently through releases on infestations of the lima bean pod borer, *Etiella zinckenella* (Treit.), in California, and the pea moth, *Laspeyresia nigricana* (Steph.), in Washington (Webster, 1936). *Macrocentrus* was recovered from *Melissopus latiferreanus* (Wlsm.) infesting the Catalina cherry in California and the filbert in Oregon (Dohanian, 1942).

The work of finding the host most suitable for the mass production of *Macrocentrus* was greatly facilitated by having a large supply of the parasite available when needed.⁷

One of the lepidopterous species most readily obtainable for experimental work and one which could be produced in large numbers under laboratory conditions was the potato tuber moth, *Gnorimoschema operculella* (Zell.). This moth has been notorious as an insectary pest ever since Smith (Branigan, 1916) first developed the potato-sprout method for mass culture of mealybugs as food for the coccinellid *Cryptolaemus montrouzieri* Muls. The moth often

⁷ H. W. Allen, in charge of the Moorestown laboratory of the United States Bureau of Entomology and Plant Quarantine, generously fulfilled all requests for *Macrocentrus*.

was unintentionally produced in large numbers when precautionary measures were not taken to prevent its introduction into the insectaries.

In the fall of 1942 Smith suggested the possibility of using the tuber moth as a host for *Macrocentrus*. The experimental work started at Riverside with the planting of potatoes early in 1943. Shipments of *Macrocentrus* from Moorestown began in May, 1943. *Macrocentrus* females from the first shipment were placed in a cage containing potato sprouts infested with tuber-moth larvae obtained in March from the Los Angeles County Insectary. This cage was located in a basement room of the Entomology Building, Citrus Experiment Station, where the light intensity was low and the temperature was about 70° F. About 30 days later 3 females and 1 male emerged from the cocoons of the tuber moth.

Since the host larvae develop in potato tubers as well as in the potato tops, there were set up three glass battery jars with cloth tops containing infested tubers and *Macrocentrus*. The parasite reproduced as readily on infested tubers as on infested sprouts. It was then apparent that a food medium suitable for the efficient mass culture of the tuber moth, and consequently of *Macrocentrus*, was at hand, a medium that could be obtained and economically handled in large amounts and one that could be held in storage over long periods of time (Flanders, 1943).

On November 5, 1943, a mimeographed article entitled "A method for the mass production of *Macrocentrus ancyliivorus* Roh." was sent to interested parties. The following February the method, considerably modified, was described by Finney, Flanders, and Smith (1944).

OUTLINE OF GENERAL PROCEDURE IN MACROCENTRUS PRODUCTION

The sequence of events from the deposition of the host eggs to the shipment of parasite cocoons requires between 20 and 30 days at a temperature of 82° F and relative humidity between 40 and 60 per cent. This sequence and the correlated life cycles of host and parasite are shown graphically in figure 1.

Mature egg-sized potatoes are superficially perforated with punctures about 1 cm apart and then placed in a single layer on a hardware-cloth tray. Infestation of the potatoes with newly hatched larvae of the tuber moth is obtained by covering the potatoes closely with a sheet of cloth having on its underside moth eggs that have been evenly distributed over the surface at the time of oviposition 3 days previous.

Young host larvae after entering the punctures are subjected to parasitization by placing the tray in a closed compartment with mated females of *Macrocentrus* for a period of 3 to 5 days. Then the trays are removed and placed in tiers above a shallow wooden container, which catches the full-fed host larvae as they leave the potatoes and migrate downward. Strips or ridges of finely crushed rock (plaster sand) on waxed plywood or sheet-metal plates are placed within the container to provide a place for the cocooning of the host larvae. The cocooning plates bearing the newly formed host cocoons are removed from the container daily. The parasite cocoons and host pupae 6 days later are freed of the host cocoons and then separated so that host-free parasites are packed and shipped into the field.

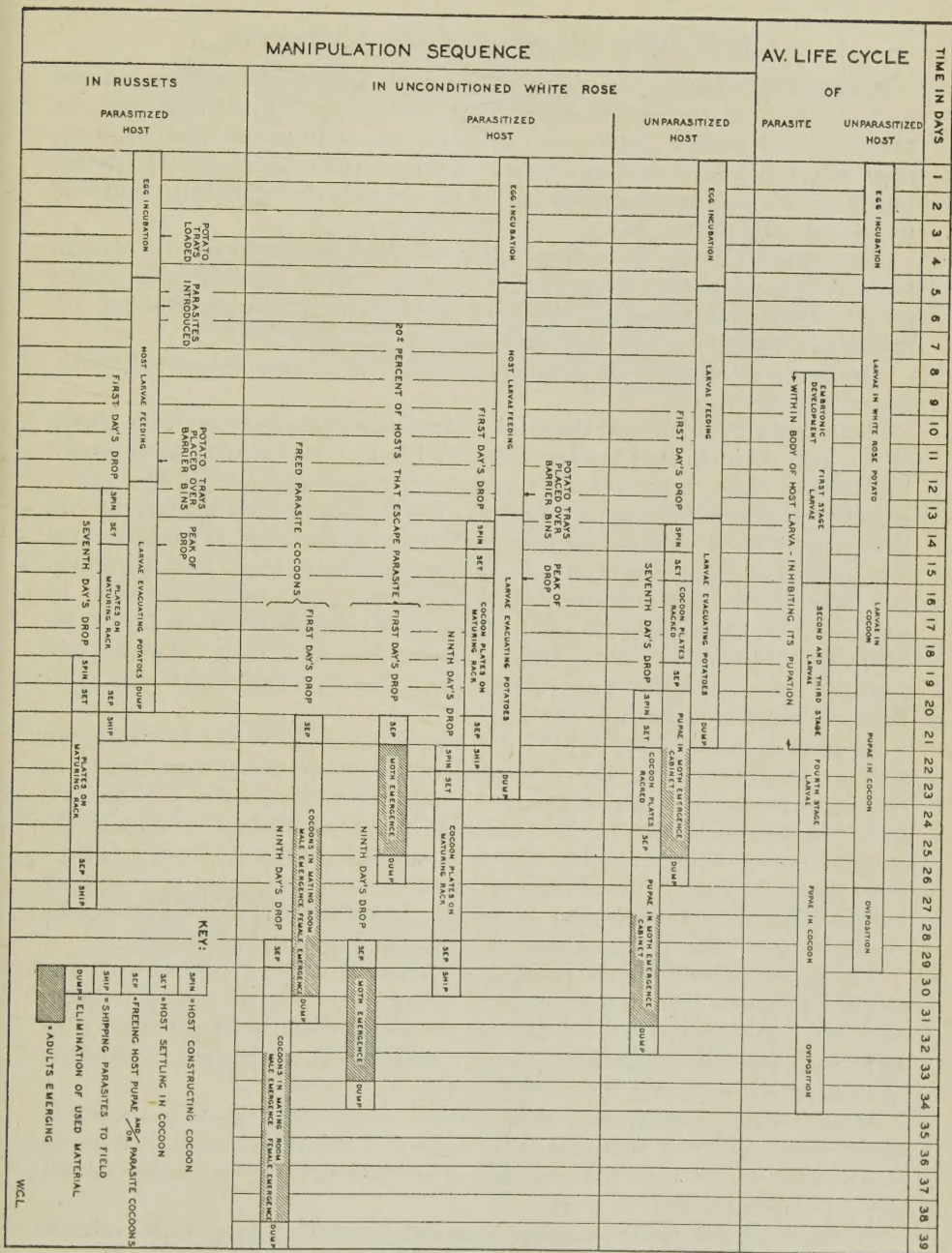


Fig. 1.—Synchronization chart of host and parasite development at a constant temperature of 82° F. Host material is equal in age but markedly unequal in development. Under certain conditions there are many slow-developing individuals, which are discarded in maintaining economy of operation.

BIOLOGY OF HOST AND PARASITE

Potato Tuber Moth. The potato tuber moth, like *Macrocentrus*, appears to be indigenous to the Americas. This is indicated by the fact that the favorite food plants, potato and tobacco, are indigenous. Furthermore, more effective insect enemies occur in America than elsewhere. The distribution of the potato around the world has been rather generally followed by the tuber moth.

The adult moth (fig. 2) is gray with a wing expanse of little over half an inch. It is most active during warm nights. During the day the adults seldom fly unless disturbed and then their flight is short and jerky. On alighting they slither into dark crevices.

The sexes occur in equal numbers. Mating takes place soon after emergence from the cocoon.

Under natural conditions the moth as a rule deposits its eggs on the leaves of the host plant so that the larva begins life as a leaf miner. As observed by Picard (1913), a roughened surface stimulates oviposition. The egg is elliptical in shape ($0.45 \text{ mm} \times 0.30 \text{ mm}$). The female deposits between 80 and 200 eggs. At 80° F and 50 per cent relative humidity, most of these are laid within 3 days after emergence. At this temperature the incubation period is 5 days. During this time the egg changes from pearly white to yellow. In potato tubers the feeding of most of the larvae ceases about 10 days after hatching. The full-fed larvae leave the tuber and spin their cocoons. They pupate 4 days later. The pupal period lasts about 9 days, so that the usual life cycle at 80° F is about 28 days.

As pointed out by Graf (1917), the larval instars after the first molt are very irregular even when food and temperature are quite uniform. Larvae of the same age may be either full-grown or half-grown. Graf noted that the rate of development seemed to be affected by the quality of food rather than by the amount. Larvae in leaves and stems develop more rapidly than those in tubers. Also larvae in mealy tubers develop more rapidly than those in nonmealy tubers. This differential when in tubers, however, may be influenced by crowding (see "The Potato Tuber in Host and Parasite Production").

The variation in host development is the principal cause of variation in parasite development. *Macrocentrus* is so well adapted to its hosts—the strawberry leaf roller, the oriental fruit moth, and the potato tuber moth—that the peak of its pupation coincides with the peak of adult moth emergence, irrespective of inherent differences in the host's life cycle.

Macrocentrus ancylivorus. When adult, the parasite (fig. 3) is a wasplike insect rusty-yellow in color and about equal to a mosquito in size. The female is somewhat larger than the male and is characterized by possessing an ovipositor about as long as its body.

In the field, the adult parasites frequent vegetation likely to be infested with host larvae. When in condition to oviposit, the female explores the surface of the plant with her antennae until she contacts the webbing or frass of a host larva. She then unsheaths her ovipositor and uses it to tap the plant surface in the vicinity until the hole containing a host larva is located. The hole is explored with the tip of the ovipositor. If she contacts a larva the ovi-

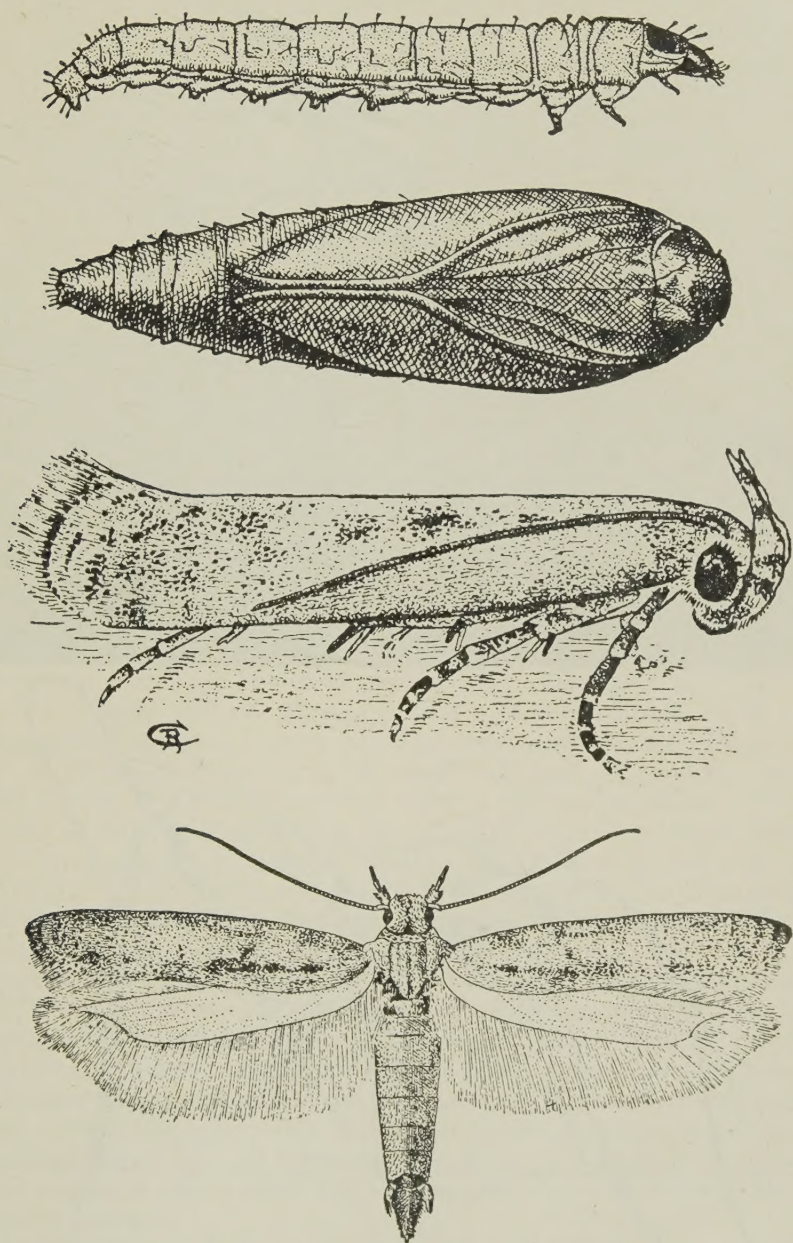


Fig. 2.—Larva, pupa, and adult of the potato tuber moth, *Gnorimoschema operculella* (Zell.). (After Graf.)

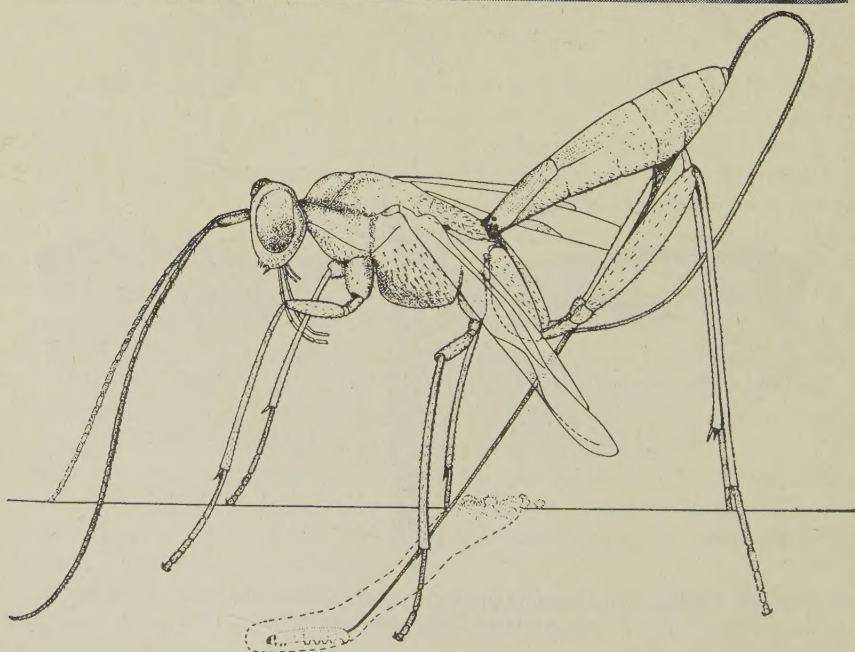
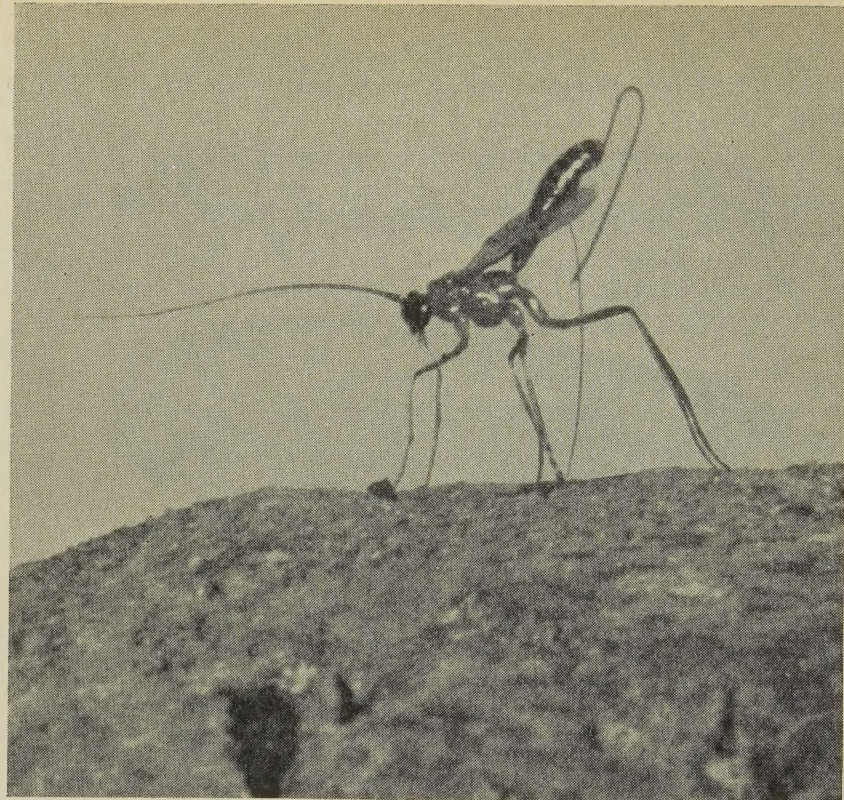


Fig 3.—*Macrocentrus* female: above, searching for the host burrow; below, in position to inject an egg into body of host. The host is attacked only while in its burrow. (Drawing after Garman and Brigham, 1933.)

positor quickly pierces its skin (fig. 3) and injects into its blood a minute egg, which is about $0.10\text{ mm} \times 0.02\text{ mm}$. As soon as the egg is immersed in blood it begins to absorb food and to grow. As Daniel (1932) has shown, however, instead of maintaining itself as a single unit, it splits polyembryonically into a number of independent parts when about 24 hours old. These consist of a preëmbryo and several pseudoembryos. Only the preëmbryo completes development, becoming a first-stage larva about 5 days after egg deposition. The number of first-stage larvae in a host depends entirely on the number of eggs deposited. But the host is superparasitized when more than 1 egg is deposited, for only one parasite can complete its development in a single host.

Macrocentrus females are capable of ovipositing about 24 hours after emerging as adults (provided the relative humidity exceeds 30 per cent and the temperature is above 60° F). One *Macrocentrus* female may be capable of depositing over 500 eggs. The maximum number of progeny of one female reared to maturity on potato-tuber-moth larvae at Riverside was 462. The female from which this record was obtained mated once and was kept at a temperature of 80° F and a relative humidity of 55 per cent. She oviposited every day for 20 days and then died of old age. The ovaries were dissected and found to be filled with eggs. Many of them, however, showed signs of disintegration. It is interesting to note that during the first 10 days the female produced 230 female and only 86 male offspring, whereas during the last 10 days she produced 146 males and no females. The first 6 days of oviposition yielded 184 females and 6 males. Such a ratio as this latter may not be desirable from the standpoint of mass culture, although 1 male has been observed to mate with 30 females during a 24-hour period. As in many Hymenoptera, unmated females produced only male offspring. Under optimum conditions *Macrocentrus* females mate only once and produce offspring having a ratio of 2 females to every male. A once-mated female produced, each day of life, progeny having the following ratio of males to females: 0 to 5; 9 to 35; 8 to 29; 6 to 19; 8 to 35; 13 to 25; 3 to 20; 6 to 11; 8 to 30; 11 to 22; 8 to 21; 7 to 11; 12 to 18; 8 to 12; 6 to 19; 0 to 4; 4 to 6; 2 to 0. The progeny developing from each day's deposition of eggs had on the average a difference of 7 days between the shortest and longest life cycles.

Macrocentrus adults emerge from their cocoons for the most part between 2 and 7 p.m. Females reach their peak of oviposition about a week after emergence; the maximum number of eggs deposited in 1 day as measured by the number of progeny was 69 (in this case 17 were males). Since *Macrocentrus* females readily superparasitize their hosts, the actual number of eggs deposited probably is considerably in excess of progeny reaching maturity. Observations indicate that the larger the host at the time of attack the greater the percentage of eggs wasted. The progeny per female decreases as the size of the host increases.

In mass-culture work, *Macrocentrus* females are utilized in parasitization for only 3 days. They are then discarded, although they have deposited only a fraction of their eggs. For the first 3 days of her oviposition period the average female deposits about 25 eggs per day (this number is based on progeny per female when 20 females were given quantities of fresh hosts daily). Eggs deposited within a 24-hour period by a female do not develop

at the same rate under constant temperature conditions. In one case a difference of 17 days occurred in the life cycle of two individuals developing at 80° F from eggs deposited on the same day. The developmental rate of the parasite is modified by the developmental conditions of the host and the amount of superparasitism.

Under mass-culture conditions the host larvae are attacked 1 to 5 days after they hatch. In such hosts the life cycle of the parasite averages about 23 days, that of the male being 1 day less than that of the female. When hosts of the same age group are attacked at different times and at different stages, the parasites tend to emerge as adults at the same time. Thus the development of *Macrocentrus* from eggs laid in full-fed hosts becomes synchronized with the development of *Macrocentrus* from eggs laid in very immature larvae. This is because the parasite does not develop beyond the first instar until the time arrives for the host to spin its cocoon. Development of the parasite from then on requires an average of 13 days at 82° F.

Observations of the life cycle of each of over 4,000 *Macrocentrus* at 80° F show that in hosts that were parasitized within 3 days after hatching, the minimum and maximum life cycles of the parasites in 70 broods^a averaged 20 and 27 days respectively, the shortest life cycle being 17 and the longest 34 days.

In the broods from hosts that were parasitized 6 days or more after hatching, the minimum and maximum life cycles averaged 18 and 25 days, respectively, the shortest being 14 and the longest 33 days.

In females that were fully impregnated, a greater proportion of the eggs were fertilized when deposited in the smaller hosts than when deposited in the larger hosts. The percentage of females from small hosts was 75, whereas that from larger hosts was 60.^b Since the parasite contacts the host only with the tip of the ovipositor, the qualities of the host are not likely to have a differential effect on the action of the spermathecal gland and consequently on the sex ratio. The rate of oviposition probably influences the fertilization of the egg. This indicates that the intervals between ovipositions are greatest when the host is small.

The *Macrocentrus* larva develops through three stages within the host larva. According to Daniel (1932) the *Macrocentrus* larva slips out of the third-stage skin and the host larva at the same time. It then turns and feeds externally on its host, finishing in about an hour. (If this host larva had not been parasitized it would have pupated 3 days earlier.) After resting for about a half hour the parasite begins to spin its cocoon within the host cocoon. The parasite cocoon is completed in about 24 hours and consists of three layers of silk forming a capsule about 5 mm × 2 mm. When first formed it is white, changing in a few hours to glistening brown. Within a few hours after constructing its cocoon the larva pupates, forming a pupa 4.7 mm × 1.6 mm. The pupal period is 7 days. The adult may live 2 days at 80° F without food or water. Mating rarely, if ever, occurs at temperatures below 55° F (Martin and Finney, 1946b).

^a A brood as used in this paper consists of all the individuals developing from the eggs deposited by a female during 1 day.

^b Superparasitism under mass culture may obscure the proportions of eggs fertilized (see under "Superparasitism," page 473) since the most abundant sex is the one that survives.

CRITICAL FACTORS AFFECTING MASS CULTURE OF HOST AND PARASITE

Confining the Host

Confinement of the host insect so that its activities can be controlled to suit the requirements of the project is necessary in the mass culture of beneficial insects. Since the parasite spins its cocoon within that of the tuber moth, it is necessary to control the activities of the latter in order to utilize the former.

In 1944 a production unit in the form of a box enclosing a tray was used. Four hundred such units were constructed (Finney, Flanders, and Smith, 1944). Each unit encompassed the production operations of infesting the potatoes, parasitizing the tuber-moth larvae and concentrating the parasitized larvae on sand-covered cardboards. Since the units were to be stacked in such a way as to confine the insects, a tight fit was required, and this necessitated the use of lumber that had a minimum amount of warping. Redwood was at first decided upon, but in the experimental units of redwood the parasites died without reproducing. Experiments proved that redwood was poisonous to the parasites. White pine and sheet iron were used instead. White-pine boxes and metal trays are to be preferred. Galvanized metal trays need not be painted.

In 1945 the production units were used only for infesting and parasitizing, the trays of infested potatoes being removed and stacked in the open rooms where the concentrations of larvae in the sand could be removed every 24 hours. This was possible as a result of the development of a barrier for confining the host larvae. This change cut down the number of boxes needed by two thirds and practically eliminated the problem of excess moisture (Finney, Martin, and Flanders, 1945).

Regulation of Temperature

Since insects are cold-blooded animals, their activities are to a large extent regulated by the temperature of their environment. For maximum reproduction under insectary conditions, temperatures should be used that give the highest rate of reproduction—that is, the quickest turnover of material. Once the temperatures have been established for each operation, they must be held constant so that a daily routine can be followed which will permit the most efficient use of labor and equipment. A temperature of 82° F has proved suitable for the mass culture of *Macrocentrus* and its host, although the potato tuber moth seems to attain its maximum size at lower temperatures. In order to avoid the cost of cooling, the insectary should be situated in a region where the daily outdoor temperatures rarely if ever exceed 82° F for more than an hour or two.

Use of Sand by Host Larvae

The larvae of the potato tuber moth are extremely active when they become full-fed and ready to spin their cocoons. They have a strong tendency to wander and to spin these cocoons out of reach. It was observed, however, that if these wandering larvae happen to encounter loose soil or silt they

will immediately spin their cocoons, attaching them to whatever serves to support the silt provided the layer of silt is not over $\frac{3}{16}$ inch thick. This habit, often observed in insectaries where potato sprouts were cultured, suggested that if the units containing the tuber-moth larvae were provided with cardboard floors covered with silt, the cocoons would be attached to the cardboard and would be under constant control and convenient to handle. This proved to be one of the most important discoveries in the evolution of this method of moth culture; the whole operation centers on this reaction of the tuber-moth larvae to silt, sand, or other similar material.

Various types of cocooning media, organic as well as inorganic, have been tested. Organic materials such as ground walnut shells have the advantage of light weight but react with the hypochlorite solution used in dissolving the cocoons of the host, producing heat and releasing chlorine. If ordinary soil is used, the cocoon material becomes muddy in the bath. It also contains objectionable organic material.

Sand composed of uniform-sized, smooth and spherical particles is not satisfactory, since when incorporated in the host cocoon it is not as quickly released upon immersion in the hypochlorite solution as is sand derived from crushed rock.

The Potato Tuber in Host and Parasite Production

The potato, if adequately infested, is completely utilized within 20 days at 82° F and 60 per cent relative humidity. This allows a rapid turnover of production material and thus conserves time and space and forestalls losses due to diseases and mites.

The efficient use of the tuber moth as a host of *Macrocentrus* requires the attainment of (1) the maximum number of full-fed larvae per pound of potatoes, and (2) the maximum uniformity in rates of development of larvae of equal age.

Given potatoes equal in surface area and number of punctures and infested with equal numbers of newly hatched larvae, the numbers of full-fed larvae produced vary with the variety of potato, the region or type of soil in which it was grown, the degree of maturity of the potato, and the evaporative power of the surrounding air. The most satisfactory grade of potato is small U.S. No. 1 harvested after the vines mature in areas where bacterial rot is least prevalent.

At a constant temperature and at relative humidities ranging between 40 and 70 per cent, the rate of larval development varies with the variety and size of potato, and the number of the larvae per potato. The rate is retarded if, because of too few punctures, there is excessive competition for food, or if the population is too low for optimum development. In a large potato as well as in a very lightly infested potato, larval development is so relatively slow that many larvae are lost when the potatoes are discarded. This is especially true when newly harvested White Rose potatoes are used.

At a temperature of 82° F the Russet potato will yield a much greater number of full-fed larvae than a White Rose potato of equal size and age. The larvae in the Russet mature a day earlier than in the White Rose. These varieties belong to different types of potatoes which apparently correspond

to two culinary types, a mealy type such as the Russet and Green Mountain and a nonmealy type such as the White Rose and Earlsine.¹⁰

When newly harvested or cellar-stored Russet and White Rose potatoes are used, there is a marked difference in the pith evident 2 weeks after being lightly infested. Then the pith of the Russet is crisp and easily broken whereas that of the White Rose is leathery and tough. The most important difference, however, is in the behavior of the larval population. In the Russet the population permeates the pith, completely utilizing the tuber; whereas



Fig. 4.—Frass or excreta of developing tuber-moth larvae pushed out onto surface of potatoes through punctures.

in turgid White Rose it remains for the most part in the cortex just under the potato skin. Consequently the host population that the latter can support is relatively small. This difference, however, is much less marked when White Rose is held for 2 or 3 months in artificial cold storage, where it is subjected to dehydration. Unless they are well conditioned, White Rose produce larvae inferior in health and vitality. Consequently they are more easily affected by adverse environmental conditions and by disease than are larvae from Russet potatoes.

More frass appears to be thrown out onto the surface of the potato by the larvae in White Rose (fig. 4) than by those in Russets. This may be an effect of excess moisture in the larval tunnels. It has been observed that larvae infesting Burbank (semimealy) potatoes do not throw out their frass if the air is dry but do so if the air is humid. It is possible, however, that dry air causes the frass near the surface of the potato to harden and thus pre-

¹⁰ A good mealy potato will consist of about 25 per cent dry matter and 75 per cent water. As dry matter decreases in potatoes, soggyiness will increase. The range in specific gravity may be from a mean of 1.100 to 1.060 (Akeley and Stevenson, 1944). Experience has shown, however, that in the ordinary run of potatoes there is such a wide range of specific gravity that flotation is useless as a means of separation.

vents its ejection. Parasitized larvae throw out less frass than unparasitized larvae. In White Rose the host larvae tend to remain near the surface or come to the surface to throw out frass where they are exposed to attack (fig. 3) to a greater extent than in the Russet.

Macrocentrus females oviposit most readily at high humidities. Probably humidities are higher in the compartments containing infested White Rose than in those containing Russets, other things being equal. As a rule the percentage of parasitism in Russets is less than in White Rose although the actual amount of parasitism may be the same.

The White Rose potato is produced in great quantities and is available over a long period of time. If properly infested and conditioned, it yields the highest number of *Macrocentrus* per pound of potato ($200 \pm$ parasites per pound) with 75 to 80 per cent parasitism of host. It improves with storage; artificial cold storage reduces weight but greatly increases the capacity for producing the host. In old White Rose, wrinkled and softened by dehydration as a result of cold storage, the relative amount of parasitism is decreased, equaling the newly dug Russets from the standpoint of infestation and parasitism ($170 \pm$ parasites per pound; 65 to 75 per cent parasitism of host).

The suitability of White Rose, therefore, as a medium for the development of the tuber moth, measured by the increase in survival, improves with the age of the tuber (improvement is slow if held under damp conditions). This change in suitability is accompanied by a decrease in percentage of parasitism, since the actual amount of parasitism per unit is not increased. In the case of the Russet potato the suitability of the tuber for tuber-moth development is not appreciably increased by aging. As noted previously, the dehydration of Russets brings about a decrease in parasitism, both in amount and in percentage.

In order to obtain the maximum number of larvae per pound of potato, small-sized tubers must be used since they have the greatest surface area for use in infesting. Egg-sized potatoes have proved most suitable for insectary use. For efficiency in production the potato must be utilized within the shortest possible time. The feeding rate of larvae in small potatoes is greater than in large potatoes since the former lose moisture more rapidly and thus become more favorable for larval development. A high initial infestation seems to increase rate of moisture loss (Finney, Martin, and Flanders, 1945). In order to obtain such an infestation the entire skin surface of the potato must be perforated with properly spaced punctures which provide suitable points of entry for the newly hatched larvae, the number of punctures equaling at least one third of the maximum number of mature larvae that the potato can support. The dehydrating action of a heavy infestation promotes feeding toward the center of the tuber.

The diameter, shape, and depth of the punctures apparently influence the amount of infestation, but of these only depth influences the amount of parasitism. The ideal type of puncture seems to be a hole formed at right angles to the surface of the potato, the hole being 1.5 mm in diameter at the surface and 3 mm in depth. Such punctures do not tend to become closed as do slit punctures and do not become impenetrable because of the formation of heavy scar tissue.

The exposed raw tissue of the potato hardens within a few hours to form a thick scar tissue (periderm), tougher than the potato skin, so that infesting should follow puncturing as soon as possible. The manner in which the tuber-moth larvae penetrate the plant tissue and the accessibility of the larvae to attack by *Macrocentrus* appears to be determined in part by the extent of the scar tissue within the puncture. Potatoes which have had the surface scarified or in which the punctures are slitlike yield fewer *Macrocentrus* than those in which the punctures are simple round holes of small diameter such as recommended above.

The cut tissues of the Russet and old, withered White Rose potatoes seem to form scar tissue more rapidly than the newly harvested or turgid White Rose. The scar tissue on the latter, however, becomes harder and more resistant. In turgid White Rose tubers the newly hatched moth larvae appear to work into the tissue at the top of the puncture, possibly because the bottom of the puncture is too moist. In Russets, however, the larvae may penetrate the tissue at the bottom of the puncture and then work upward behind the scar-tissue walls of the punctures, where they would be more or less protected from attack by *Macrocentrus*. *Macrocentrus* does not thrust its ovipositor through plant tissues, even the most delicate. The manner in which the potato skin is cut when punctured appears to influence both the entry and the parasitization of the tuber-moth larvae. Some of the skin should be torn away.

Apparently during the early larval stages of the host it is the thickness and extent of scar tissue rather than the rapidity of the inward migration of the larvae, as suggested by Finney, Martin, and Flanders (1945), that protects them from attack by *Macrocentrus*.

The area of exposed potato flesh in the puncture may influence the number of larvae infesting the potato.

INSECTARY BUILDING REQUIREMENTS

The requirements of a building suitable for the mass culture of the potato tuber moth and its parasites are not exacting if it is located in a region where the maximum daily temperatures rarely exceed 82° F and where the relative humidities at 82° F do not exceed 60 per cent. The Albany insectary (fig. 5) is in such a location. The building should be so insulated that a constant temperature of 82° F can be maintained in the rooms at a minimum of expense.

The equipment used in production requires a relatively large amount of space for housing and handling. The ventilation equipment should be sufficiently effective to eliminate excessive heat due to solar radiation. The stratification of heated air in the production room must be prevented by adequate air circulators.

A division of the insectary into six rooms is necessary for convenience of operation. The operation and temperatures of these rooms are as follows:

1. Room for storing enough potatoes to last for at least 2 weeks; outdoor temperatures.

2. Room for the operations of puncturing potatoes, loading trays and placing them in the incubation units, and unloading and cleaning trays; temperature 70° F.

3. Room for infesting potatoes with tuber moth, parasitizing tuber moth with *Macrocentrus*, cocooning of host and parasite, housing of egg-deposition boxes, emerging of adult hosts, and incubating egg sheets. This room should be as large as the other rooms combined, without windows, with a temperature of 82° F and relative humidities of from 40 to 60 per cent according to the water content of potatoes.

4. Room for separating host and parasite and packing of parasite cocoons for shipment; temperature 75° F.

5. A small, low-ceilinged room with a window on the north side for the collecting of *Macrocentrus*; temperature 70° to 75° F. This is the only room in which a window is necessary. Philip H. Marvin in correspondence states that



Fig. 5.—Insectary building located at Albany, California. The production capacity of equipment installed in this building is over a quarter of a million parasites daily.

artificial light may be used to attract the adult parasite. A collecting screen of white cloth, about 15 inches in front of the window, is fastened to walls, ceiling, and floor.

6. Room for office work, files, and so forth; temperature about 72° F.

7. A shed for housing tray sterilizer, washing rack, and trays not in use is also necessary.

PROCEDURES AND EQUIPMENT IN PRODUCING HOST AND PARASITE

Selection and Handling of the Potato Tuber

Handling Conditioned and Unconditioned Potatoes. The potatoes used for the production of the tuber moth and *Macrocentrus* should be egg-sized U.S. No. 1 grade. Potatoes grown in light sandy loam are preferable since usually they are not washed and consequently hold up well.

A mealy-type potato such as the Russet is recommended for general insectary use. Since potatoes suitable for the efficient propagation of tuber moth and *Macrocentrus* are not available throughout the season, arrangements should be made for holding the needed amount in storage for several months; the storage temperature should be about 38° F.

Russet potatoes are harvested during the fall months in the Tehachapi and Tulalake regions and may be held there at a very low season-storage rate. In late March they should be transferred to artificial cold storage. Russet potatoes keep well and may still be suitable for use during July or even until mid-August, when a new crop is harvested. It is advisable to remove potatoes from storage at least 2 weeks before infesting them.

To obtain the best results from White Rose or other nonmealy potatoes they should be thoroughly mature when harvested. This may account for the fact that the White Rose from Bakersfield, harvested early during the hot season, are much less satisfactory than White Rose from Tulalake. Nonmealy potatoes stored under artificial refrigeration seem to improve in quality more rapidly than when stored in underground cellars or caves.

It is essential to have a continuous and adequate supply of moths for infesting purposes in the insectary. These moths may be obtained either from those escaping parasitization in the parasite units or from unparasitized units devoted exclusively to moth breeding stock. When White Rose are used, particularly potatoes that are unconditioned (newly harvested), the number of moths escaping parasitization cannot alone be depended upon for infesting purposes. Hence about 15 per cent of the trays loaded daily are used solely for the production of moth breeding stock. Parasitism is low in Russets, especially if they are old and conditioned. For this reason during the major part of the season, if Russets are used, no units for moth breeding stock need be set up; all may be used for the production of parasites. The percentage of parasitism, as well as the total number produced per tray, is less variable in Russets than in White Rose. Because of this variation, when White Rose potatoes are used, more units and trays must be used solely for moth breeding stock than may be actually necessary. Since the host larvae develop more rapidly and more uniformly in Russets, the tuber is completely utilized in 16 rather than in 19 days. The more rapid turnover permits the use of over 25 per cent more trays than with White Rose in the same floor space.

The production units are used most efficiently when Russets are infested, since the Russet supports relatively large numbers of tuber-moth larvae which mature in a relatively short time with relatively little variability and relatively low percentage of parasitism. The actual amount of parasites reared per tray per generation may not equal that when White Rose are used, but the total per tray per season will be greater.

When White Rose are used, a total of 84 units can be set up with the facilities of the Albany insectary. If the White Rose are unconditioned (freshly dug) 20 of these units would have to be used for moth breeding stock leaving 64 for parasite production. These could be expected to produce about 2,000 parasite cocoons per unit, or 128,000 per day. The parasitism would range from 80 to 90 per cent. If the White Rose were well conditioned, at least 10 units would have to be devoted to moth breeding stock, leaving 74 for parasites. These would produce at best an average of 3,000 parasite cocoons per unit, or about 222,000 per day. The parasitism would average 65 to 75 per cent.

When, instead of White Rose, Russets are used, a total of 108 units can be set up with the same amount of floor space. Newly dug Russets may produce

3,500 parasite cocoons per unit, with parasitism of 60 to 70 per cent. If well conditioned, they will produce about 3,000 per unit, or about 325,000 per day; and parasitism will be 30 to 45 per cent. In either case there should be enough unparasitized moths to provide adequate infesting stock. This expanded production with Russets of course entails the use of more potatoes, and also a greater number of moth-oviposition units, potato trays, and incubation units. This probably would be worth while if a large production early in the season is considered important.

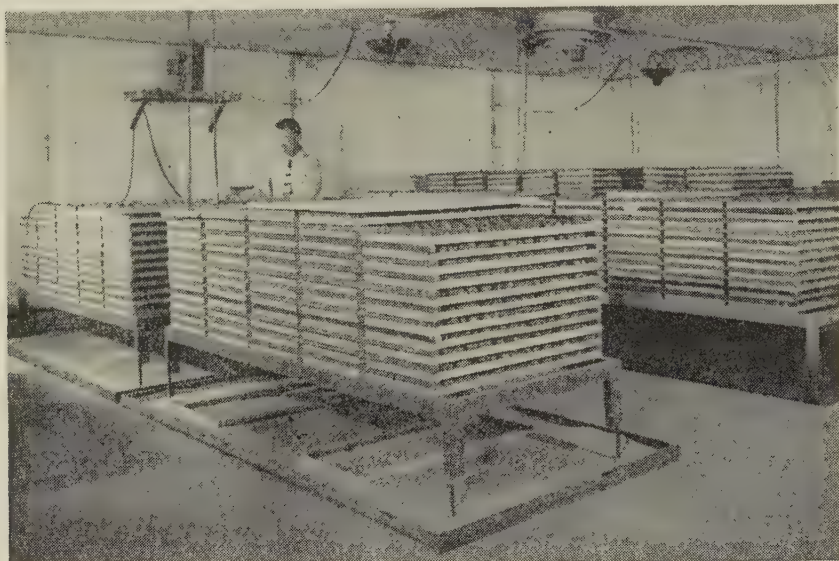


Fig. 6.—Stacks of loaded potato trays on racks set within barriers. Note cocooning plates on floor of containers. Position of hot-wire barrier in container in foreground is outlined by the white silk formed by the migrating larvae.

Bliss Triumph potatoes were given a brief trial with good results. The parasitism was between 70 and 80 per cent and the average production between 2,500 and 3,000 parasite cocoons per unit. Some extra units would be required for moth breeding stock. Triumphs did not prove to be good keepers. They might occupy a niche between the old Russets and the new crop in late summer production, since they are harvested in May in California.

Potato Puncturing Equipment. The potatoes are punctured to facilitate the entrance of the maximum number of tuber-moth larvae. The potatoes should be infested as soon as possible after the punctures are made. At present the potatoes are punctured by rolling them on a nail-studded board with a tack-studded leather mitt.

The board is a 5×8-inch piece of $\frac{3}{4}$ -inch 5-ply plywood with 1-inch nails about 1.5 mm in diameter driven through it. The nails should form a pattern of 1-cm squares over an area 4×6 inches. In the mitt, the leather holding the tacks is 4½×9 inches, about $\frac{1}{8}$ inch thick and fairly stiff. The tacks used are cut carpet tacks long enough to protrude about 5 cm out of the leather, forming a pattern of 1-cm squares over an area 3½×6 inches. A second piece of

leather identical in size to the first is cemented over it so as to cover the heads of the tacks and anchor them firmly. The two are then stitched together around the edges. A soft leather "pocket" about one half the length of the leather pad is sewn on the top to hold the operator's fingers. A strap with a buckle is sewn across the lower end to fasten around the operator's wrist. When puncturing the potato the mitt should be manipulated with a rolling motion. The potato is placed at the end of the board farthest from the operator, then the "heel" of the mitt is placed on the potato and pulled so that the potato rolls across the nail-studded surface.

An automatic puncturing machine has been developed and is ready for use as soon as it can be modified so that it makes the proper type of puncture.

The Potato Tray. The punctured potatoes are poured onto trays (fig. 6) made of $\frac{1}{2}$ -inch hardware cloth fastened to a metal frame. The operator should endeavor to arrange the potatoes on the tray with as few as possible touching. This spacing, which is to prevent larvae from forming cocoons between the potatoes, is done by hand and can be very time-consuming if one is too meticulous. The potatoes remain on these trays until discarded. The trays should be so formed that there are no sharp angles or narrow crevices that will stimulate the formation of cocoons.

Infesting and Parasitizing

The Incubation Unit. Infesting of the potato and parasitizing of the larva of the tuber moth take place in closed boxes or incubator units. These units (fig. 7) are constructed so that they can be stacked snugly one on the other, the bottom of one unit then forming the top of the one below.

A unit may be made either of nonresinous wood or sheet metal. It should be large enough to contain a tray of potatoes with $\frac{1}{2}$ -inch clearance on all sides and at least 1-inch clearance between the layer of potatoes and the bottom of the tray above.

When in use the units need to be ventilated. This is accomplished by means of louvers in the long sides of each. Each louver is about $1\frac{1}{2}$ inches wide and extends the length of the side. It is covered with gauze fastened to the box with waterproof resin glue such as Weldwood or Cascamite. The gauze retains the proper amount of moisture (60 to 70 per cent relative humidity) within the incubator unit to secure the maximum amount of oviposition. One end of each unit is provided with a hole about $1\frac{1}{8}$ inches in diameter for the purpose of removing the egg sheets and introducing the female *Macrocentrus*. The hole is closed by a cork or cloth stopper.

The loaded potato trays are held in the incubator unit for 8 days, during which period the infesting and the parasitizing are completed.

Infesting of the potatoes with newly hatched larvae is accomplished by means of a cloth sheet (figs. 8, 9), upon one side of which the moth adults have uniformly distributed their eggs.

The trays in use in the Albany insectary hold 20 pounds of potatoes apiece. Since 20 pounds of potatoes will support an average of 7,000 to 9,000 tuber-moth larvae, the egg sheet should carry about twice that number of eggs. An excess of eggs tends to reduce the differential in infestation between the potatoes at the edge of the tray and those at the center.

A day or two before the moth eggs are due to hatch the egg sheet is placed, egg side down, on a tray of freshly punctured potatoes within an incubation unit, which is then placed in a stack.

The egg sheet should be large enough so that it touches all the tubers in the tray. Tubers that are not in contact will have few if any larvae in them. The sheet should be lightly pressed between the potatoes with the fingers. Larvae



Fig. 7.—Incubation units in which infestation and parasitization occur. The apertures through which the egg sheets are removed and parasites introduced are closed with cloth-covered cotton plugs. Each platform carries two stacks of incubation units.

that drop between the potatoes to the bottom of the incubation unit either perish or eventually find their way up the sides of the tray where they enter the first potato they encounter. This may account, in part, for the heavier infestation at the edges of the tray.

One or 2 days after the eggs begin to hatch the potatoes are well infested. The egg sheet in the units to be devoted to parasite production is then removed by means of a pair of long forceps through the aperture in the end of the incubation box; the honey strips are laid in, and the parasites introduced. The honey strips are prepared in advance by drawing a heavy streak of honey

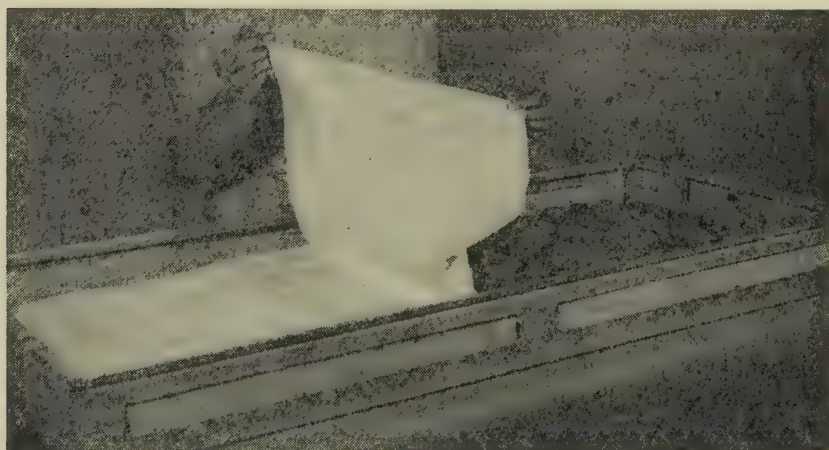


Fig. 8.—Placing egg sheet on potato tray in incubation unit. Most of the eggs hatch within one day after being placed on potatoes.

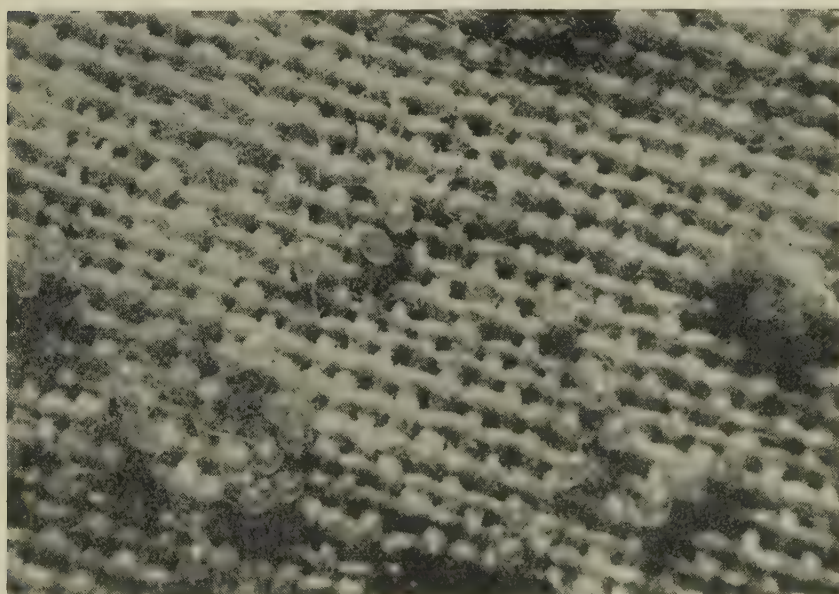


Fig. 9.—Portion of used egg sheet magnified to show cloth texture. Note tuber-moth eggs.

the length of a strip of waxed paper ($1\frac{1}{2} \times 10$ inches) and then covering it with another strip. When the honey strips are to be used, they are separated and then partially folded lengthwise so that they can be inserted into the units through the aperture in the end. One strip is placed on the potatoes in each unit.

With food thus provided, most of the parasites will live until the stacks are

dismounted. Without food they will die within 2 or 3 days, according to the amount of moisture in the box.

The incubator unit is stocked with mated females of *Macrocentrus* at the rate of 5 females per pound of potatoes. This should be done (when Russets are used) immediately after the egg sheets are removed.

When White Rose potatoes are used the parasitization period is limited to about 3 days in order to minimize the injurious effects of excessive super-parasitism. This is accomplished by delaying the introduction of parasites until 3 to 4 days before the units are to be dismantled. When the trays are moved to the racks, the parasites are allowed to disperse through the room.

Migration and Cocooning of Full-fed Host Larvae

One day before the larvae begin to leave the tubers, usually the ninth day after the egg sheets are placed on the potato trays, the incubation units are taken down and the trays placed on a rack set within a barrier.

Each rack should be large enough to hold all the trays loaded daily. Several stacks of trays may be placed on a rack. In order to facilitate the migration of the larvae and to provide suitable ventilation, the stacks of trays on each rack should be separated by about 2 inches. The trays on each stack should also be separated to allow the larvae to crawl out between them. At the Albany insectary the ends of the trays are supported by smooth sticks 1 inch square and long enough to extend beyond the length of the racks and to support the ends of several stacks of trays. The trays when stacked must be directly above one another so that the migrating larvae dropping from the edges of the tray fall directly to the barrier floor.

The trays remain on the rack for 8 to 10 days, according to the type of potatoes being used. With unconditioned White Rose potatoes, at least 10 days are required for the larvae to become full-fed and vacate the tubers; with Russets, 8 days. At an operational temperature of 82° F, using White Rose, 12 racks are sometimes needed—10 racks for trays holding parasitized infestations and 2 for unparasitized infestations.

Tray-Rack Construction. The rack (fig. 6) should be strong enough to carry the weight of trays and potatoes stacked upon it. The number of angles and niches which might induce cocooning by the full-fed larvae should be kept at a minimum. Iron-pipe construction suspended from ceiling would probably make an ideal rack. The ceiling support would allow free and unobstructed access to the cocooning plates below. There will be very little if any travel up the pipe since the larvae tend to migrate downward. The ceiling will need to be strong enough to support the load suspended from it. The stirruplike frame could be constructed so it might easily be taken down when not in use.

The rack if not suspended is provided with legs about 16 inches in length, supported by the floor within the confines of the barrier. The legs should come to a point where they rest on the floor, and if the barrier is of wood construction a small iron plate should be placed under the point to protect the floor. The points provide a minimum of contact on the floor within the barrier so that comparatively few larvae will find their way up the legs and reënter the potato trays.

Any angles or niches on the rack where the larvae tend to spin cocoons should be coated with paraffin so that the cocoons can easily be removed. This coating can best be applied by painting with paraffin-benzine solution (2 parts of saturated solution of paraffin in benzine and 1 part of benzine).

Construction of Container for Supporting Barrier and Enclosing Cocooning Plates. Under each rack is a container for trapping the larvae dropping from the trays above. The container should extend at least 10 inches beyond



Fig. 10.—Cocooning plates showing ridge rows of tuber-moth cocoons embedded in sand.

the rack on all sides to catch the larvae which flip outward from the stacks of trays when disturbed by one another. The floor should be constructed of 3-ply plywood, treated with linseed oil. The walls should be constructed either of Oregon pine or white pine, about 2 inches high and $\frac{3}{4}$ inch thick, and nailed and glued securely to the floor. The glue should be waterproof. The angle between the floor and the wall should be rounded to prevent cocooning there. Curved blocks should be set very solidly into each corner, eliminating any sharp angles. The curve should have a 45° arc with a 5-inch radius.

The Hot-Wire Barrier. A barrier consisting of Nichrome resistance wire fastened by small staples is placed on the inside of the container walls. A gauge of wire should be used that will be heated to about 180° F when connected to a 110-volt line. The barriers of two containers may be connected in series if desired. A rheostat may be desirable to make possible fine adjustment of the current. The wire should not be laid on the container floor because

many larvae might be injured by flipping against it. The wire should be secured about every 2 inches and run in a slightly zigzag manner. This tends to allow for contraction of the wire when it is disconnected for cleaning. The wire should be at the desired temperature while being installed. The staples should be placed so that as much as possible of their exposed surface will be in contact with the wire. A cold staple acts as a bridge for the escaping larvae.



Fig. 11.—Mat of cocoons carefully seraped from cocooning plate exposing contents of many cocoons.

At the Albany insectary 23 feet of 18 B & S gauge Nichrome wire is used on each container and two are connected in series, making a total of 56 feet. When connected to a 110-volt circuit, the wire is heated close to the proper temperature.

The Cocooning Plate. The cocooning plates (fig. 10) are simply sheets of well-seasoned 3-ply plywood slightly longer and wider than the potato trays. The edges should be beveled so there is no sharp angle between the plate surface and the barrier floor. They should be made of dry wood so as not to warp. Each side should be painted with two thin coats of the paraffin-benzine solution, and aired well 2 days or more before they are used. Strips of dry sand about 2 inches wide and not more than $\frac{1}{2}$ inch thick and about $\frac{1}{2}$ inch apart are spread across the plate from side to side. The amount of sand on each plate and the number of plates used is dependent upon the number of larvae expected to drop on that particular day. The larvae will fill the strip solidly if it is not more than $\frac{1}{2}$ inch deep (fig. 11). If more sand is needed later in the day

it can be added on top of the strips already there. Judgment in the amount of sand to be used will be developed by experience.

No. 2 Monterey beach sand is used at the Albany insectary. "Plaster sand," such as is available at most rock crushers, may be used. It should be sifted through 18-mesh screen if 16-mesh screen baskets are used in the separation process. Sand can be used repeatedly if allowed to aerate and dry thoroughly.

The cocooning plates after being loaded with sand are carefully placed within the barrier. The larvae should be gently swept aside with a soft-bristled counter brush before a plate is laid on the floor.



Fig. 12.—Host-parasite separation equipment. Note basket containing mass of cocoons removed from cocooning plates.

Each day the cocooning plates on which the sand is matted together with cocoons are removed from the barrier and placed over the stacked potato trays above, in order to allow any unsettled larvae to return to the newly spread sand on the plates below.

The next day, after the larvae have become firmly established in their cocoons, the plates are removed, stacked, and set aside for a 6-day period. During this period the parasite larvae leave the host larvae and spin their cocoons within the host cocoons.

Separation of Host and Parasite by Sodium Hypochlorite Process

On the morning of the seventh day after cocooning, 75 per cent of the parasites on the cocooning plates will have pupated, and the host cocoons and their contents are run through the sodium-hypochlorite-alcohol separation process in order to obtain *Macrocentrus* free from moths (Bartlett and Martin, 1945; Finney, 1945).

The silk solvent used in the separation process is made up of 1 part of a stock solution of 21 per cent sodium hypochlorite with 6 parts of water. If

21 per cent sodium hypochlorite is unavailable, a commercial bleach, containing 5.25 per cent sodium hypochlorite, bottled for household consumption, may be used—1 part bleach to 1 part water. The alcohol used is isopropyl and when diluted with water has a specific gravity of 0.92 (not Baumé). Dilution is 3 parts of 99 per cent isopropyl alcohol to 2 parts of water.

The equipment consists of three earthenware crocks (fig. 12), one for the solvent solution, one for tap water, and one for alcohol; two wire baskets (fig. 14), one of which fits loosely within the other, made of bronze welding rods and 16-mesh copper screen; a number of cloth drying frames; and an electric fan.



Fig. 13.—Moth pupae and parasite cocoons in bottom of wire baskets after having passed through the sodium hypochlorite solution and having been separated in alcohol.

The smaller of the wire baskets is equipped with a rigid bail over the top and with a bottom divided in half to form two flaps hinged on the middle line and opening downward. The flaps are manipulated by a jointed Y-shaped rod, the stem of which extends above the top of the basket where it is hooked over the bail.

The procedure in separating host and parasite is as follows:

The matted cocoons are lifted from the paraffined plates with a piece of sheet metal (beveled on the lifting edge) and placed in the small basket to a depth of about 3 to 4 inches. The basket is lowered into the solvent crock until the cocoons are deeply immersed. The basket is next raised slowly a few inches in the bath with a slight twisting action, then lowered quickly, so that the material is given a gentle toss and roll in the liquid. In about 45 seconds to 1 minute the host cocoons are completely dissolved and the freed sand has dropped to the bottom of the bath. The small basket, now containing only the parasite cocoons and the larvae and pupae of the host, is shifted to the vat of tap water where the material is rinsed for 10 seconds. After rinsing, the basket is lifted free of the water and allowed to drain thoroughly. Then

the small basket is set within the large basket placed in the alcohol crock, the small basket being elevated so the bottom flaps will hang vertically when released. As the host pupae sink, the bottom is opened and the surface of the



Fig. 14.—Close-up of wire baskets to show construction.

bath gently stirred. All the viable host pupae as well as any larvae of both host and parasite sink to the bottom of the large basket while the parasite cocoons float within the small basket.

The small basket, with bottom closed, is removed from the alcohol and its contents of parasite cocoons dumped on the cloth drying frames (fig. 15). This process of separation is completed in about 20 to 30 seconds (fig. 13).

The mass of parasite cocoons is spread out thinly to dry in a current of air



Fig. 15.—Frames holding parasite cocoons and host pupae in drying rack—
one day's production.

from the fan. The large basket containing a mixture of host pupae and some larvae of host and of parasite is then removed from the alcohol, and its contents dried on cloth sheets.

The solvent solution must be used only when it is in a stable condition; that is, when there is no free chlorine being produced. Decomposition of the solution begins after a certain amount of organic material has accumulated in the vat. This organic material is derived from the silken cocoons, dead host larvae, and larval frass from the potatoes. Decomposition is indicated by the production of chlorine accompanied by a frothy scum. There also is an increase in the time required for dissolving the host cocoons and an increase in the temperature of the solution. Sufficient quantities of chlorine may be released to irritate the mucous membranes of the operators (Martin and Finney, 1946a).

The pupae and larvae of the tuber moth, the full-fed larvae of *Macrocentrus*, and the cocooned larvae and pupae of *Macrocentrus* can be passed through the separation process without mortality. This process has been used with equal success with several other parasites of the potato tuber moth.

Mortality of *Macrocentrus* while in the parasite cocoons averages about 18 per cent with a range from 5 to 25 per cent.

The unparasitized material from units devoted to moth breeding stock may be processed 3 days earlier than the parasitized material. Although no separation is required, drying is facilitated if the host pupae are dipped in the alcohol bath.

Handling of Host Pupae and Adults

The host pupae after separation are placed in a shallow wire basket of 16-mesh screen. If there are too many moth larvae among the moth pupae, they should be separated by flotation in a saline solution having a specific gravity of 1.05.

The baskets are set in a gastight cabinet (fig. 16), where the adult moths emerge. The cabinet should have 30-mesh screen across the top to provide ventilation but should be closed by a tight-fitting lid when anesthetizing. At the Albany insectary about 20,000 pupae (200 grams) are placed in each basket.

Anesthetizing Moths. The moths are readily handled in quantities if temporarily rendered inert—stupefied. The use of ether was suggested by Smith for this purpose. Anesthetizing of the moths is quickly accomplished by exposing them to a mixture of ether and carbon dioxide fumes. When carbon dioxide is passed, at $1\frac{1}{2}$ pounds' pressure, over liquid ether in a half-filled 1,000-cc Erlenmeyer flask, the resulting mixture is nonexplosive—it will, in fact, actually extinguish a flame. A small pressure regulator with a scale calibrated from 1 to 50 pounds should be used. It should be enclosed in a metal container heated with a light bulb so that it will not become iced and cease to function properly. The dial should extend outside this box so that it may be read easily. The ether should be kept near room temperature by immersing the flask in lukewarm water. If this is not done, the flask will become iced and not enough ether fumes will be picked up by the carbon dioxide.

Two cylinders of carbon dioxide should be connected together so that a low or fluctuating pressure in one, when it is nearly exhausted, can be compensated

for by the opening of a full one. This makes it possible to completely exhaust a cylinder and still retain a constant working pressure.

The air in a moth-emergence cabinet will have been replaced by carbon dioxide and ether after a 5-minute injection of this gas. A 15-second flow is



Fig. 16.—Moth-emergence cabinets, one opened to show roofed containers for holding moth pupae.

sufficient for each egg-deposition box; a 20-second flow is necessary on a box-cleaning day in order to keep the moths inert the extra period of time.

Two to 3 minutes after the air in the cabinet has been replaced by the fumes of carbon dioxide and ether, anesthetizing is complete. The drawer at the bottom is removed and the moths are poured into a trap-bottomed screen basket similar to that used in the separation process. A current of air should be directed upward through the bottom of the basket with a force sufficient

to mildly agitate the moths and carry away loose moth scales and yet not spill the moths over the side. The cleaned moths can then be gently dropped through the trap door into a container having an ether-soaked sponge attached to the underside of its lid. The moths may be held inert for several minutes until ready for use.

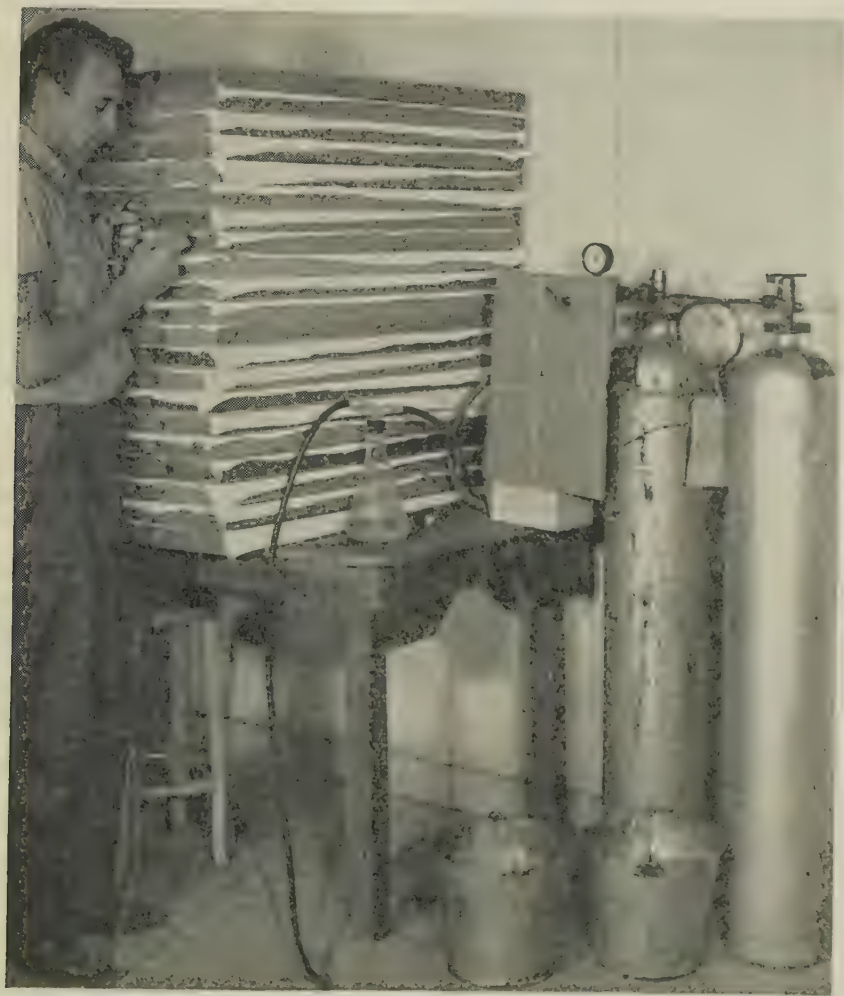


Fig. 17.—Equipment for mixing ether and carbon dioxide and injecting mixture into egg boxes.

The moths are measured into glass tubes calibrated to the amount required for each egg-deposition box. The tubes are 6 inches in length and 1 inch in diameter. One end is covered with a fine-meshed screen cap; the other is open except when the tube contains moths, at which time it is plugged with a cloth-covered cotton wad.

Construction of the Moth-Emergence Cabinet. The moth-emergence cabinet is a plywood box large enough to hold trays containing six 1-day allotments

of moth pupae for insectary propagation stock. The upper four fifths of one side is made to form a door. The top is covered by a 30-mesh bronze screen and is normally open except during the anesthetizing process, when a snug-fitting plywood lid is used to retain the carbon dioxide and ether.

The box is set on legs equipped with casters so that it may be easily rolled from room to room. The legs should be long enough to raise the box to a convenient working height. A hole $1\frac{1}{8}$ inches in diameter is drilled near the top of one side to permit the introduction of the anesthetic gas. It is at other times closed with a cork. At the bottom of the cabinet is a metal drawer into which the moths fall when anesthetized. The front of the drawer is made of wood

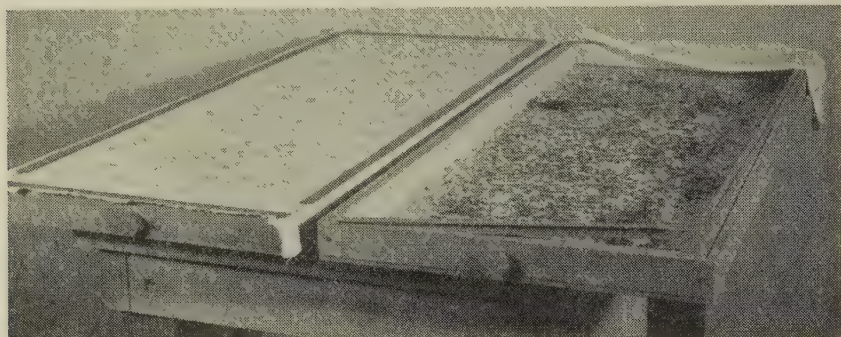


Fig. 18.—Close-up of egg boxes, to show method of fastening egg cloth. One is opened after moths have been anesthetized.

and when closed occupies most of the remaining fifth of the space below the door. The drawer has a flange in two sides and back made to slide in grooves constructed in each side and the back of the cabinet. This prevents moths from crowding into the area around the sides and underneath the drawer.

The cabinet is equipped with rods for supporting the pupa trays. Each tray is covered with a removable steep-pitched sheet-metal roof so that, during the process of anesthetizing, the moths will not fall back into the trays but will be deflected and drop on down to the drawer.

Construction of Egg-Deposition Box. An egg-deposition box (fig. 18) has white-pine sides $1\frac{1}{2}$ inches high, a plywood bottom, and a removable cloth top. In one end is an opening 1 inch in diameter closed by a cork. The box should be tight enough to hold carbon dioxide. The inside of the box should be painted with white enamel paint. The top surface of the sides of the box are grooved to form narrow slots into which T-shaped metal strips are fitted, one strip to each side. The cloth top is held in place by being wedged into the slots by the metal strips. The cloth is drawn taut in the process. The cloth top consists of bleached muslin and is 3 inches wider and longer than either the egg box or potato tray. Allowance should be made for shrinkage when the cloth is laundered. It should be laundered rough dry and each sheet should be laid flat to avoid creasing and wrinkling.

Various materials have been tested for use as egg sheets, including paper toweling, blotting paper, and unbleached muslin. Suitable material must have the oviposition surface covered with a nap that stimulates oviposition, must be

strong enough so that it can be drawn and held taut with a smooth, perfectly horizontal surface, and flexible enough so that it can be tucked around the potatoes in the production trays. Bleached muslin such as no. 60 longcloth has proved most suitable.

In the daily removal and replacement of the egg sheets, it is necessary to anesthetize the moths within the egg box. This is accomplished by injecting the carbon dioxide and ether mixture (which is under $1\frac{1}{2}$ pounds' pressure per square inch) to displace the air in the box.



Fig. 19.—Egg-box chambers, one opened to show position of egg boxes while moths are laying eggs on egg cloth. Note two trays containing wet burlap for maintenance of 80 per cent humidity.

The steps in changing the egg sheets are as follows:

1. Boxes are removed from the egg-box humidity chamber.
2. Each box is gently upended and then turned sidewise, vertically, to concentrate dead moths in one corner.
3. Boxes are stacked one on top of the other to confine the carbon dioxide and ether mixture.
4. Carbon dioxide and ether are injected into one box after another, each box receiving gas for 15 seconds.
5. As soon as the moths in a box are "down," the egg sheet is removed and stacked (not more than 15 sheets to a stack).
6. Dead moths are removed from the corner of the box by a tank-type vacuum cleaner and the gas residue lightly fanned away.
7. A new egg sheet is stretched and clamped into position.
8. Additional moths (1.5 moths per square inch of cloth surface) are blown out of glass tubes into the boxes by air current from the compressor.

9. Egg boxes are replaced in the lightproof chamber (fig. 19), in which relative humidity is about 85 per cent.

The total number of moths in each egg box is held at about 10 moths for each square inch of the cloth top, provided the relative humidity is 80 per cent or higher.

The moth debris, which stimulates oviposition by the moths, is usually removed twice a week with a vacuum cleaner after the stupefied live moths have been poured into clean egg boxes. When this is to be done the moths are anesthetized with a 20-second application of the gas.

Handling of *Macrocentrus* Cocoons for Propagation Purposes

The *Macrocentrus* cocoons to be used for propagation in the insectary are placed in screen-bottom trays like those used for moth pupae but without the roofs. The trays are placed in a lighttight emergence cabinet, the same as that used for moth emergence. They remain in the cabinet until emergence is complete (about 10 days at 84° F). From 85 to 90 per cent of the cocoons should produce parasites if they have been reared on healthy host larvae and properly handled during the separation process. With a sex ratio of 1 to 1, each 1,000 cocoons should yield 400 females. Sometimes insectary temperatures become unusually high, causing many parasites to emerge ahead of schedule. Then when the temperature lowers there is a lag in emergence. Therefore it is advisable to allot a few more cocoons than the number indicated above, so as to be assured of sufficient inoculation stock each day.

Since *Macrocentrus* cocoons have a fairly constant average size when potatoes of one variety and uniform condition are used, they can be measured by volume quite successfully. But with any change in potato type there will be a change in average cocoon size which makes it necessary to recalibrate the measuring device.

Each day the cabinet is rolled into the collecting room where the door is opened and the parasites are permitted to fly to the window of the room. The females for use in propagation are collected by suction into glass tubes identical to those previously described for holding anesthetized moths to be blown into the moth egg-deposition boxes. One hundred females (no males) are collected in each tube (fig. 20). After the necessary number of females has been collected, the remaining females and males can be removed by means of a tank-type vacuum cleaner.

Before the cabinet is closed and returned to its position in the insectary, a tray containing empty cocoons is removed and one containing cocoons most recently separated is put in its place. The temperature of the room may be a compromise between a point that is comfortable to the operator and one at which the insects will be sufficiently active to leave the cabinet readily. The use of a small feather duster will assist in the evacuation of the cabinet.

At 75° to 80° F the progeny of females that emerge and mate average from 40 to 47 per cent females. The progeny of parasites emerging and mating at 84° F would be approximately 55 per cent females. Lorin Anderson, operator of the State Insectary at Palisade, Colorado, reports over 63 per cent females as a result of temperatures above 84° F during periods of warm summer weather.



Fig. 20.—Collecting *Macrocentrus* females into tubes for transfer to production units. (Courtesy California Department of Agriculture.) Below, a section of screen magnified.

The proportion of eggs fertilized tends to increase with temperature when the interval between the emergence of the females and their collection is relatively short. This effect may be the result either of increased mating action or of more rapid transfer of sperm from spermatophore to sperm capsule (see the next section).

Humidity should be supplied by placing wet sphagnum moss in the drawer at the bottom of the cabinet. A 30-mesh screen on a frame capable of being easily removed for cleaning should be placed above the drawer to prevent empty cocoons or parasites from falling onto the wet moss where an unsanitary condition might develop.

FACTORS AFFECTING THE SEX RATIO

The parasite cabinet is darkened to reduce the frequency of contact between sexes. In the absence of light the parasites distribute themselves more or less uniformly. The population usually is most dense on ventral surfaces. Frequency of contact when the cabinet is dark is sufficient for the mating of the maximum number of females. With increased light intensities there is an increased amount of moving about, particularly by the males, and consequently an increased amount of mating action. Then many females mate more than once. A male may mate 30 times or more. Multiple mating tends (1) to prevent the impregnation of the females, that is, the storing of sperm in the spermatheca, and (2) to prolong the preoviposition period.

The females of *Macrocentrus ancyliivorus* normally refuse to mate more than once. But under relatively high intensities of daylight, multiple mating occurs commonly, provided the density of the parasite population is such as to permit frequent contact. As Voukassovitch (1926) observed in *Macrocentrus abdominalis*, the "multinuptae" female during the first mating remains quiet, whereas in subsequent matings she moves about, dragging the male, and often tumbling and rolling. Under field conditions it is probable that a female rarely, if ever, mates more than once. An unimpregnated female produces only male offspring.

Population of once-mated females produce progeny predominantly female, the likely ratio being 1 male to 2 females. Holloway (1934) found that the usual ratio under field conditions was 2 males to 3 females. The greater proportion of males in the field may be the effect of oviposition preceding mating. When laboratory conditions are optimum for impregnation, the numbers of females produced should outnumber the males nearly 2 to 1. In one test 60 impregnated females produced 2,846 offspring having a sex ratio of 1 male to 2 females.

When a female mates, it receives from the male a relatively large spermatophore, which must be discarded before an egg can be laid. At a temperature of 80° F, 6 hours are required for the transfer of all of the sperm from the spermatophore to the sperm capsule. At 84° F this transfer probably is completed in less time. This would mean that when the collecting-room temperature is kept at 84° F a greater proportion of the parasites are ready to oviposit at the time of collection than when the operating temperature of the room is 80° F or lower.

If the spermatophore is not emptied at the time of collection it may be dis-

lodged during the process so that the female does not receive a full supply of sperm.

Multiple-mated females may contain 2 to 10 spermatophores or more. Nevertheless, half of such females that oviposit may produce only male offspring.

The preoviposition period of such females may be prolonged several days (usually less than 5 days) because of the additional time required to discard excess spermatophores. Females in which the preoviposition period exceeds the period allowed for parasitizing in the inoculation units produce no progeny.

Once-mated females may not oviposit for 12 hours after mating because of the presence of the spermatophore. As high as 20 per cent of ovipositing once-mated females may have only male progeny, either because they are unimpregnated or, if impregnated, because their spermathecae do not respond to stimuli from the host.

SUPERPARASITISM

Sometimes, when unconditioned White Rose potatoes are used, as many as 4 per cent of the *Macrocentrus* larvae are lost because they fail to cocoon before being run through the separation process. It is inadvisable to attempt to save this 4 per cent because separation too long delayed results in loss of moths due to emergence before separation and in shortening the shipping period. Superparasitism may be one of the causes of late cocooning. Few superparasitized larvae complete their development. These die and turn black and therefore are discounted. At the end of the 3-day parasitization period 50 per cent of the host larvae may contain from 2 to 28 first-stage larvae of *Macrocentrus*.¹¹

Eight to 10 days are required for the parasitized host larvae to evacuate the potatoes completely. With White Rose potatoes the percentage of hosts parasitized as they leave the potatoes may vary from 39 on the first day, to 56 on the third day, 72 on the seventh day, and 93 on the tenth day. With Russet potatoes the percentage parasitized may vary from 20 on the first day to 85 on the fifth day.

The average ovipositing female deposits about 20 eggs per day at a constant temperature of 80° F so that during the 3-day period of parasitizing, when the inoculum consists of 100 females, as many as 6,000 eggs may be deposited in the larvae infesting the potatoes on a single tray.

Superparasitism by once-mated females if not excessive may be advantageous since it tends to increase the proportion of females (Martin and Finney, 1946b). Competition between the sexes occurs only during their first instars. Since there are more females than males in this competition the females are more likely to survive. But when the inoculum includes unimpregnated females, superparasitism may be very disadvantageous because of the excess of males eggs deposited (Flanders, 1945a).

Prolongation of larval development resulting from superparasitism may account for the fact that among the progeny of a female that oviposited in 24 hosts in a 24-hour period, there was a 16-day differential in life cycles at a constant temperature of 80° F.

¹¹ These data and those in the following paragraph are from unpublished observations by Charles H. Martin.

PACKING, SHIPPING, AND COLONIZING

Small paper bags (size SS $\frac{1}{2}$) are used for the shipment and liberation of *Macrocentrus* (fig. 21). A loop of string is attached to the top of each bag so that it can be suspended in the field.

The cocoons of the parasite are poured into the bags, about 1,000 per bag. A loose wad of excelsior is next placed in the bag on top of the cocoons. The bag is closed by folding the top. It is then fitted snugly into a shipping container.

Each day a sample of 100 cocoons is set aside for rearing in order to obtain the sex ratio and per cent emergence. The value of *Macrocentrus* releases depends, in part, on the proportion of females.



Fig. 21.—Equipment for packaging parasite cocoons for shipment.

Since *Macrocentrus* disperses readily, the bags are placed on every tenth tree. Before suspending the bag, it is opened and the wad of excelsior pulled up to keep the top open. Many of the adults crawling through the excelsior are able to rid themselves of their cocoons, to which they would otherwise have remained attached. *Macrocentrus* begins to emerge from its cocoon about 5 days after passing through the separation process if held at 82° F. Adults may continue to emerge for a week or more after the cocoons are placed in the field, the period depending on existing temperatures. They are placed in the field at the rate of 2,000 per acre per week.

In regions of frequent rainfall it may be necessary to use bags of cheesecloth. The bags should be placed where the danger of attack by hyperparasites or predators is the least.

The transportation of *Macrocentrus* from insectary to orchard, often over distances of several hundred miles, is an important phase of the work. In the beginning of the project the cocoons of the parasite were shipped while within the host cocoons. It was practically impossible not to include a few tuber moths. Although there was no real harm in this, it seemed better to avoid

the shipment of pests if for no reason other than that it might be misunderstood by some orchardists.

It was found that anesthetized adults of *Macrocentrus* could be held under refrigeration for 4 days without injury. The shipment of such adults, however, required rather expensive insulated shipping units which were not readily obtainable (Smith, 1944). The problem of transportation was solved by the development of the host-parasite separation process, which permitted shipment of the parasite while still in its cocoon, free of its host, and over long distances (Bartlett and Martin, 1945).

As Garman and Brigham (1933) have shown, *Macrocentrus* does not oviposit at temperatures below 60° F and is sluggish in action at temperatures below 65°. This habit may limit the successful utilization of *Macrocentrus* to regions where the temperatures during the greater portion of the time are above 65°.

SANITATION

Sanitation measures rigidly enforced will prevent losses from such pests as the mite *Pediculoides* sp., the chalcid *Dibrachys* sp., the red disease, and the black disease (Steinhaus, 1945).

The most effective measure is the rapid turnover of material and its proper disposal afterward. All discarded potatoes, before they are carried away to a dump, should be fumigated with methyl bromide in a chamber large enough to accept one day's disposal.

The hot-wire barrier if properly placed and cared for is 100 per cent effective in preventing larvae from escaping and spinning up in crevices where they may be found by *Pediculoides* or *Dibrachys*.

The potato trays are cleaned by immersion in boiling water (fig. 22) until the cocoons, dried frass, and other debris are sufficiently loosened to be readily removed through the flushing action of water under pressure. At the Albany insectary, the trays are boiled for 15 minutes. The hot-water treatment used in cleaning the potato trays is also effective in eliminating material in which pests may breed.

Dry-cleaning the trays with a stiff-bristle brush was found to be slow and unsatisfactory.

The red and black diseases of the tuber-moth larvae cannot be completely eliminated but may be reduced to less than 1 per cent by the use of Russet potatoes or well-conditioned White Rose free from rot. More than 85 per cent of the moth pupae obtained are viable. The red and black diseases may be curbed by preventing high humidities in the cocooning room where larval feeding is at its peak. Temperatures above 87° F in the incubation or cocooning rooms will cause an outbreak of the red disease. If this temperature should persist, the loss due to this disease could be serious. High temperature seems to be the prime factor causing epidemics of the red disease.

In the late fall and winter of 1946-47 there was a lowering of the percentage of emergence of both moths and parasites and also a reduction in the percentage of parasitism. During February the host emergence dropped to nearly 50 per cent and parasite emergence was down to between 65 and 70 per cent. Material examined by E. A. Steinhaus showed that 45 per cent of larvae still within the potatoes contained microsporidian spores. Of the evacu-

ated larvae 20 per cent contained spores, a fact which indicates that many infected larvae perished within the tuber. Protozoa were found in 20 per cent of the host pupae and 20 per cent of the parasite larvae and pupae.

Several sheets of eggs were treated by immersing the eggs for 20 minutes in water heated to 118°–119° F (Allen and Brunson, 1947). When eggs were treated, 95 per cent of host pupae emerged, as contrasted with 50 per cent from untreated eggs. Host production rose from less than 4,500 per unit to more than 10,000 per unit. From cocoons reared on hosts from heat-treated eggs, 89 per cent of parasites emerged, as compared with 64 per cent from hosts reared from untreated eggs.



Fig. 22.—Dipping potato trays in hot-water sterilizer to destroy pests and loosen any cocoons that adhere to trays. Trays are placed on tilted racks for washing.

Allen and Brunson (1945) report a considerable number of adult *Macrocentrus* of both sexes attacked by a microsporidian. The heavily diseased individuals were characterized by conspicuous whitish areas on the abdomen. In such cases the abdomen becomes smaller and malformed. The life span of such an individual is shortened.

Allen and Brunson found the progeny of diseased and healthy adults of *Macrocentrus* to be equally affected. Infection apparently occurs in the digestive tract while *Macrocentrus* inhabits the host and adults may manifest it several days after emergence. As noted by McCoy (1947) the presence of the microsporidian in *Macrocentrus* "is not as a true disease but more by accident."

COST OF PRODUCING MACROCENTRUS ANCYLIVORUS

Season of 1944. Tables 1 and 2 give the figures for colonization of *Macrocentrus* for the year 1944, by counties and by months. As shown, the total number of *Macrocentrus* colonized during the year was a little over 19 million. This does not include the parasites used as parent breeding stock, or those used for experimental work. The actual production amounted to well over 20 million. The figures in the tables are estimates, but it is believed that the

possible error is not in excess of 10 to 15 per cent. At the Albany insectary the figures were secured by estimating the number of moth cocoons per cocoon sheet, and then multiplying this number by the fraction that was parasitized. The latter figure was obtained by removing 1-inch-square sections of the cocoon sheets and actually determining the fraction of moth cocoons that contained parasite cocoons. At the Riverside insectary, the adult parasites were colonized, rather than the parasite cocoons, and the number of adult parasites was estimated at the time of collection.

Collection of the parasites, from an illuminated cloth screen, into no. 12 paper bags lightly filled with excelsior, was accomplished by the use of a large aspirator having a mouth $1\frac{1}{2}$ inches across. The cloth screen was marked off in 6-inch squares, and practice made it possible to estimate with sufficient accuracy the number of parasites on each square.

The cost of producing the parasites may be considered from several different angles. Most likely to be of interest to an entomologist, however, is that cost for which, with established techniques, he might reasonably expect to be able to produce these by the thousand, omitting charges for research. The principal items entering into this cost are housing and services, equipment, salaries and wages, and supplies (primarily potatoes).

The cost of housing and of services varies so greatly, not only with the locality but with the requirements of the individual entomologist, that no figure is given here. At both Riverside and Albany, structures which have been built for other purposes were remodeled to provide the necessary conditions, such as temperature and ventilation, for use as insectaries.

The production units described in the previously mentioned article (Finney, Flanders, and Smith, 1944) were used with satisfaction throughout 1944. The cost of the complete units (box and tray) varied greatly. At Riverside, wooden units were used, and these, including painting, cost around \$7.00 each. The units used at Albany were constructed entirely of galvanized iron and cost, roughly, \$2.75 each. Most of the workers seemed to prefer the wooden units, but these certainly were not sufficiently superior to justify the extra cost.

At Riverside, 400 production units were operated; at Albany, 1,500. The total cost of these units, with which approximately 19 million parasites were produced, was about \$7,500. There is no reason why such units should not last several years. Depreciation charges should be calculated accordingly, for addition to production costs.

Labor is an important cost item in mass production of most parasites. Considerable progress has been made, however, in reducing this item by mechanization wherever possible.

Salaries chargeable to production were estimated at \$5,000. This amount would provide for an insectary superintendent and an assistant superintendent. Since the work must be done every day in the week, two men are required for supervision.

Labor costs for the production of the 19 million *Macrocentrus* in 1944 totaled, roughly, \$7,335. The labor was mostly female, at the rate of 75 cents per hour.

The principal expendable supply used in the mass production of *Macro-*

centrus was potatoes. For the 19 million *Macrocentrus*, 1,890 sacks (189,000 pounds) of potatoes were used, at an average cost, exclusive of transportation, of about $1\frac{1}{2}$ cents per pound, or \$2,835. For parasite production for orchard colonization, potatoes were used at the rate of 15 pounds per unit, an average of 63 units being loaded per day, for a period of 200 days. The low cost of the potatoes was due to the fact that so-called peewees and egg-sized tubers were found to be most suitable, and were considerably cheaper than the regular commercial grades.

The principal items in the cost of producing 19 million (table 1) *Macrocentrus* for orchard colonization in 1944 were therefore as follows:

Salaries	\$ 5,000
Wages	7,335
Potatoes	2,835
Total	<hr/> \$15,170

For these items, the cost per thousand parasites was about 78 cents. To this cost would also have to be added proportionate charges for certain miscellaneous items such as freight on potatoes, depreciation of building and equipment, supplies such as cardboard and sand or other material for cocoon sheets, and services such as heat, light, water, and telephone.

Season of 1945. The colonization of parasites reached a total of 15,935,000 in 1945, as compared with 19,295,000 during 1944. The drop in production over the previous year, as will be seen by a study of table 1, occurred during June, July, and August. This slump in production was the combined result of a number of unfavorable developments which, at that time, could not have been foreseen, as follows:

1. Failure of the supply of breeding moths. This was caused by an unexpectedly high percentage of parasitism in newly dug White Rose potatoes. This shortage of moths alone cut to one third the number of moth eggs available for the next cycle of production.
2. Use of one bad lot of cheap, unconditioned White Rose potatoes, which was heavily infected with rot and disease. Mortality was high; the few insects that reached maturity were weak and unhealthy.
3. A lapse of 5 days during which no trays were set up, because the Army had taken over all supplies of potatoes.
4. Failure of the potato-puncturing machine to create punctures suitable for obtaining high parasitism.

During the 1945 season all the *Macrocentrus* production work was carried out in one insectary, at Albany. Approximately 2,200 sacks of potatoes were used, at a cost of roughly \$5,500. A better grade of potatoes was used in 1945 than in 1944. Labor costs were somewhat greater in 1945, due partly to higher wage scales, and amounted to \$8,835. Costs per thousand *Macrocentrus* were therefore considerably higher than in 1944. But if the summer slump in production had not occurred, costs in 1945 would undoubtedly have been lower than in 1944.

Season of 1946. The total number of parasites shipped from the Albany insectary in 1946 (April to October, inclusive) was 22,935,000. The total pro-

duced was 28,812,000. This number, however, was not the maximum possible with the equipment used, since during the last part of the season the potatoes (White Rose) were not properly conditioned for maximum production. The potatoes were held in the caves at Tulalake for several months before it was realized that such storage did not properly condition them.

The value of the 1946 colonization was considerably enhanced by the fact that the number of females exceeded the number of males. This fact also means that the number of cocoons reserved for propagation purposes greatly exceeded requirements.

Production costs were somewhat higher than in the two previous years. The cost of labor was \$10,281. Of this, \$7,343 was for general assistance and \$2,938 was the salary of the insectary superintendent. There were 3,082 sacks of potatoes used at a cost of \$5,456.

PRACTICAL RESULTS

As pointed out originally, this project had two objectives: first, to develop a cheap method for mass production of the parasite *Macrocentrus ancylovorus*, anticipating the time when orchard control of the oriental fruit moth would

TABLE 1
COLONIZATION OF *Macrocentrus ancylovorus*, BY MONTHS

Month	Number of parasites colonized		
	Year 1944	Year 1945	Year 1946
	<i>thousands</i>	<i>thousands</i>	<i>thousands</i>
April.....	103	736	1,539
May.....	1,171	4,160	3,547
June.....	3,965	3,110	3,562
July.....	4,537	453	3,901
August.....	3,559	1,754	4,300
September.....	3,695	3,213	4,296
October.....	2,265	2,509	1,790
Total.....	19,295	15,935	22,935*

* The total number produced in 1946 was 28,986,000.

become necessary; second, to retard as long as possible the spread of the oriental fruit moth into the great commercial peach-producing areas of the state.

It is generally agreed that the first objective has been satisfactorily attained. It is difficult, however, to measure the degree to which the second objective has been reached. Aside from a few backyard trees in Orange County, the pest has not, up to this time, built up appreciably in the state. The extent to which the condition can be attributed to *Macrocentrus* is extremely difficult to determine, because of the scarcity of larvae of the host. The only larvae found in the commercial peach-growing areas in 1945 and consequently the only data available bearing on this point are given in table 3, compiled by Leslie M. Smith, Division of Entomology and Parasitology, University of California, from data collected by Frank Summers at Parlier, Fresno County.

TABLE 2
COLONIZATION OF *Macrocentrus ancyliivorus*, BY COUNTIES

County	Number of parasites colonized		
	Year 1944	Year 1945	Year 1946
	<i>thousands</i>	<i>thousands</i>	<i>thousands</i>
Fresno.....	899	2,766	6,490
Kern.....	3,287	2,216	2,251
Merced.....	1,610	3,572
San Bernardino.....	1,987	1,531	1,729
Kings.....	1,440	1,427	3,121
Tulare.....	4,080	1,394	1,415
Sutter.....	3,291	1,317
Riverside.....	757	1,001	781
Stanislaus.....	2,412	886	1,335
Orange.....	839	214
Los Angeles.....	1,142	812	356
Santa Clara.....	75	651
Placer.....	41	383
Sacramento.....	20	637
Total.....	19,295	15,935	22,935

TABLE 3
PARASITISM IN LARVAE COLLECTED FROM PEACH TWIGS AT PARLIER*

Date collected and orchard	Larvae in sample	Larvae parasitized by <i>Macrocentrus</i>	Larvae not parasitized
1945: July 26, Bigger.....	3	2	1
Aug. 13, Bigger.....	4	1	3
Aug. 14, Slaiker.....	4	2	2
Aug. 16, Slaiker.....	1	1	0
Aug. 20, Barr.....	6	6	0
Aug. 21, Barr.....	5	5	0
Sept. 21, Bigger.....	2	1	1
Oct. 1, Barr.....	2	1	1
Oct. 4, Barr.....	1	0	1
Total, 1945.....	28	19	9
1946: June 11, Bigger.....	3	3	0
June 12, Bigger.....	6	4	2
June 17, Bigger.....	7	7	0
June 19, Bigger.....	1	1	0
July 1, Bigger.....	18	5	13
July 3, Barr.....	1	0	1
July 3, Bigger.....	18	8	10
July 7, Bigger.....	15	8	7
July 22, Bigger.....	4	4	0
July 30, Bigger.....	4	1	3
Aug. 6, Bigger.....	16	12	4
Aug. 7, Bigger.....	26	19	7
Aug. 12, Bigger.....	6	4	2
Aug. 15, Barr.....	3	2	1
Aug. 21, Bigger.....	1	0	1
Total, 1946.....	129	78	51

* Data supplied by Francis Summers, through Leslie M. Smith, Division of Entomology and Parasitology.

ACKNOWLEDGMENTS

Several staff members in addition to the authors made important contributions in the development of methods. Blair Bartlett was in charge of the insectary at Albany from its inception to July, 1945, and was responsible for initiating the development of the process now used for freeing parasite cocoons from moth cocoons by use of a silk solvent. He also made major improvements in the design of moth oviposition units. C. H. Martin was instrumental in refining several techniques involved in parasite production as a result of detailed researches at Albany. F. A. Gunther suggested mixing carbon dioxide with ether to render the latter nonexplosive. James K. Holloway, for several years engaged in *Macrocentrus* and other parasite work for the United States Bureau of Entomology and Plant Quarantine in the eastern United States, was of much assistance as a result of his extensive experience, and he was responsible for the detailed arrangements for field colonization of the parasite by the California State Department of Agriculture and the county agricultural commissioners.

The funds for this investigation were originally appropriated to the California State Department of Agriculture by the California State Legislature, which contracted with the University of California to carry on the study. From July 1, 1945, financial support came from funds of the University of California Agricultural Experiment Station.

The late David B. Mackie, of the California State Department of Agriculture, gave enthusiastic support to the project, as has his successor, H. M. Armitage. The original stock of *Macrocentrus* was supplied by H. W. Allen, of the United States Bureau of Entomology and Plant Quarantine. L. A. Burtch, Agricultural Commissioner of Kern County, and J. S. Rowell, Agricultural Inspector, Santa Barbara County, were of material assistance in arranging for purchase of potatoes.

The early experimental work was greatly facilitated by the donation of 140 sacks of peewee and egg-sized potatoes by five potato growers in Cuyama Valley: Adolph Kirschenman, Ed Kirschenman, William Kirschenman, Earl Roscamp, and Oscar Schaeffer. The late William Kirschenman was the principal donor.

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